Towards patient-based cancer therapeutics

The Cancer Target Discovery and Development Network*

Orienting cancer drug discovery to the patient requires relating the genetic features of tumors to acquired gene and pathway dependencies and identifying small-molecule therapeutics that target them.

Small-molecule drug discovery was originally a compound-based activity. The process begins with the discovery of a biologically active compound, often a naturally occurring small molecule. The next step involves the identification of a disease that may benefit from treatment with the compound, followed by optimization and development of the eventual drug (or drugs through synthetic modifications). Penicillin is an early example of a drug that arose from this approach. Despite many advances in drug discovery in the intervening decades, compound-based drug discovery is still common today. Rapamycin (Rapamune, sirolimus), for instance, was discovered as a secondary metabolite of a *Streptomyces* strain and was explored without success as an antifungal agent before emerging as an effective immunosuppressive agent. Synthetic derivatives of rapamycin have now been approved or are being investigated as therapeutics in cancer (Torisel, temsirolimus; Afinitor, everolimus; ridaforolimus) and in other diseases.

The ability of recombinant DNA to provide nearly unlimited access to human proteins resulted in a second approach that is also common today—target-based drug discovery. Here, therapeutic targets are selected using insights gained most often from biochemistry, cell biology and model organisms. Small molecules are identified that modulate the targets (often by small-molecule screening) followed by optimization and clinical testing. Although this is a robust process, the common failure of candidate drugs in late-stage clinical testing, owing to unforeseen toxicity or lack of efficacy, reveals limits in our ability to select targets using surrogates of human physiology, such as *in vitro* assays and animal models.

Advances in human genetics suggest that a third approach—patient-based drug discovery—may offer an alternative with a lower rate of attrition when translated to human trials. Molecular characterization of patient tissues is providing remarkable insights into the root cause of many disorders. As these insights often point to targets and processes that are believed to be especially challenging for small-molecule therapeutics—targets such as transcription factors and regulatory RNAs and processes such as disrupting specific protein-protein interactions—scientists have been innovating in chemistry, cell-culture science and mechanism-of-action studies, among other fields. As a consequence, these hard-to-drug yet key targets and processes are being pursued with new optimism.

Although heritable disorders and infectious diseases are the subject of intensive patient-based drug-discovery efforts, recent insights into the genetics and biology of human cancers have made this family of diseases a prime target for this approach. High-throughput genetic, epigenetic and proteomic analyses of cancer tissues are providing unprecedented molecular insights into genes and pathways causally related to oncogenesis, tumor progression and drug sensitivity and resistance. This points to a path entailing the determination of genomic features of patients' tumors and the discovery and development of new types of therapeutics that target the dependencies (that is, addictions) arising from the specific patterns of genetic or epigenetic alterations within them[1]. This path has been validated in a growing number of extraordinary cases[2,3]. But its generalization is a tall order, one far from the reality of current routine clinical medicine and

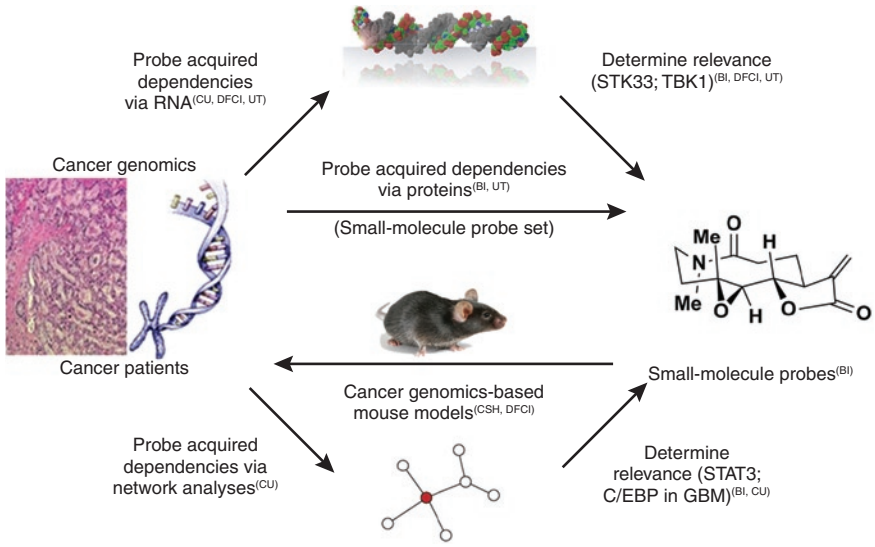

Figure 1 The NCI's Cancer Target Discovery and Development (CTD^2) Network aims to relate the genetic features of cancers to acquired cancer dependencies and to identify small molecules that target the dependencies. The centers where the approach is being undertaken are abbreviated in parentheses: BI, Broad Institute of Harvard and MIT; CSH, Cold Spring Harbor Laboratory; CU, Columbia University; DFCI, Dana-Farber Cancer Institute; and UT, University of Texas, Southwestern Medical Center at Dallas.

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not without additional challenges for payers, patients and healthcare providers[2,4].

The National Cancer Institute's approach

To pursue this path comprehensively and prospectively, the US National Cancer Institute (NCI) created the Cancer Target Discovery and Development (CTD^2) Network (http://ocg.cancer.gov/programs/tddn.asp). The Network currently comprises five interacting centers (**Fig. 1**). The mission of the CTD^2 Network is to decode cancer genotypes so as to read out acquired pathway and oncogene addictions of the specific tumor subtypes and to identify small molecules that target these dependencies. The Network builds on the data and insights gained from The Cancer Genome Atlas, Therapeutically Applicable Research to Generate Effective Treatments initiative and other cancer genomic efforts that are systematically cataloging the genetic and epigenetic alterations of specific cancers (e.g., mutational status and changes in gene expression, DNA methylation and chromosomal segment copy numbers). The CTD^2 Network is probing the consequences of these alterations on the dependencies or co-dependencies different cancers have on specific oncogenes or their interacting genes (that is, 'oncogene addiction' and 'nononcogene co-dependencies')[5]. Cataloging these Achilles' heels and linking them to the causal genetic alterations will be critically important for therapies that are personalized to individuals, including combination therapies aimed at targeting many such dependencies at once. It will also be important for anticipating resistance mechanisms and identifying clinical biomarkers.

The CTD^2 Network is currently taking five integrated approaches to determine the targets and processes upon which defined cancer genotypes become dependent. First, techniques that enable the systematic under- or overexpression of selected mRNA transcripts are being used to identify candidate genes. Second, computational network analyses are being performed on cancer genomic data sets to reveal critical master regulatory hubs in the circuitries of cancers, that act as integrators of the complex spectrum of genetic alterations that determine specific tumor subtypes. Third, a small-molecule probe set has been assembled, having members that modulate the activity of defined proteins and pathways that constitute candidate tumor dependencies. These compounds are being tested in many genomically characterized cancer cell lines, and small-molecule sensitivities are thus being correlated to the genetic features of the cancer cells. (In each of these three approaches, the CTD^2 Network measures the fitness of cancer cells having defined genetic features following targeted perturbations.) Fourth, simultaneously, probe-development projects are being undertaken to yield novel small molecules that modulate the functions of cancer therapeutic targets revealed by these approaches. Finally, the consequences of these and other agents that interfere with gene function are being, or will be, tested in, for example, mouse models of cancer having genetic alterations that closely mimic the patient-derived cancers (**Fig. 1**).

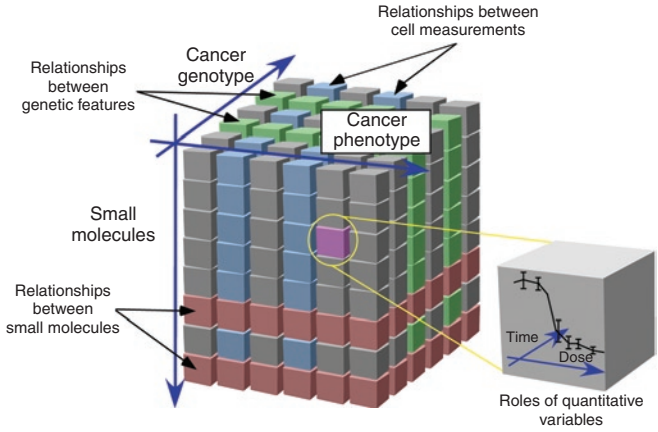

Figure 2 Conceptual image of a matrix of data relating cancer genotype, cancer phenotype and sensitivity to highly specific small-molecule modulators of cancer-relevant proteins. The CTD^2 Network is performing quantitative cellular measurements using small molecules (both with and without a knowledge of their targets) and genetically characterized cancer cell lines (copy number variation, mutational status and gene expression). Computational analyses are being performed that correlate the pattern of sensitivity with the genetic features of the cancer cell lines[9–11]. These analyses yield hypotheses for cancer genotype–drug efficacy relationships that can be tested *in vitro* and *in vivo* using systems developed within the CTD^2 Network.

Probing acquired dependencies using RNA

The CTD^2 Network is exploiting the extraordinary advances in modulating gene function using RNA interference–based knockdown or RNA overexpression methods. Three examples illustrate the principles behind this approach to identifying acquired somatic genotype–specific dependencies.

The CTD^2 center at the University of Texas Southwestern Medical Center at Dallas is screening genomic small inhibitory (si) RNA libraries against a large panel of non-small cell lung cancer cell (NSCLC) lines derived from human tumors to identify, as a particular NSCLC subtype or clade, siRNAs that are lethal only to cancers that share a similar cancer genotype[6]. Clade-specific lethal siRNAs are being used to identify metabolic vulnerabilities that occur in a particular cancer subtype, vulnerabilities that might be exploited for developing genetically matched anti-cancer therapeutics.

The CTD^2 center at the Dana-Farber Cancer Institute in Boston is screening short-hairpin (sh)RNA libraries to identify different types of cancer vulnerabilities. For example, in a screen of 20 human cancer cell lines, Barbie *et al.*[7] have looked for kinases selectively required for cell survival that depend on oncogenic KRAS and found that, second only to KRAS itself, the noncanonical kinase TBK1 was a synthetic lethal partner.

At Columbia University in New York, the CTD^2 center is using pooled shRNA libraries to complement the computational analysis of master regulators of high-grade glioma subtypes and of glucocorticoid resistance in T-cell acute lymphoblastic leukemia.

Probing acquired dependencies by network analyses

Context-specific regulatory networks of the tumor cell are being assembled and interrogated computationally to reveal otherwise cryptic master regulator proteins whose gain or loss is necessary and sufficient for tumor initiation or progression. These proteins are emerging as master 'integrators' of a spectrum of genetic and epigenetic alterations contributing to the malignant phenotype and thus provide promising novel biomarkers as well as targets for therapeutic intervention (**Fig. 2**).

For instance, at the CTD^2 center at Columbia, C/EBP and STAT3 were recently identified as synergistic master regulators of the mesenchymal subtype of glioblastoma by computational analysis of a regulatory network dissected from a large collection of gene expression profiles of human high-grade gliomas[8]. Validation was achieved by two

experimental approaches: shRNA-mediated silencing of these two genes reduced tumor aggressiveness in orthotopic xenografts and co-ectopic expression reprogrammed murine neural stem cells along an aberrant mesenchymal lineage.

Probing acquired dependencies by modulating proteins

The dramatic clinical consequences of linking genetic features of cancers to drug efficacies, including response rates of >80%, are well known, yet these advances today benefit <1% of those suffering from cancer[3]. The CTD^2 centers at the Broad Institute in Cambridge, Massachusetts, and the University of Texas, Southwestern Medical Center, are relating the genetic features of cancers to small-molecule probes or drug efficacies broadly. The CTD^2 Network is extending earlier efforts[9–11] in several ways: first, it is assembling and synthesizing highly specific small molecules (currently a collection of 225 probes and drugs) that target a wide range of proteins and that exploit advances in probe discovery[12,13]; second, it is creating small-molecule screening collections with novel chemical properties; third, it is making quantitative cellular measurements in a wide range of human cancer cell lines treated with the compounds; and fourth, it is identifying the genetic features in these cells that correlate with sensitivities of the small-molecule probes or drugs.

The CTD^2 Network is studying the novel compounds it identifies using cell lines whose genomic features (e.g., copy number, mutation or expression) have been richly characterized and parallel many of the changes found in human cancers[14,15] (although not without exception[16,17]). The intent of this effort is to identify (i) therapeutic targets of cancers linked to specific genetic features associated with cancers[10]; (ii) combinations of targets that, by using guided combination therapy, yield high rates of durable responses; and (iii) potential resistance mechanisms associated with such targets[18]. The resulting data and resources will be publicly available through the project's web site at the end of this year (http://ctd2.nci.nih.gov).

Probe-development projects for novel cancer targets

The CTD^2 Network also aims to accelerate the development of genetically matched cancer drugs by discovering novel small-molecule probes of candidate cancer targets not yet modulated by small molecules. The goal is to identify these gaps and to undertake collaborative probe-development projects involving high-throughput screening, follow-up and medicinal chemistry and biology, and mechanism-of-action studies. Advances in the science of probe discovery, especially in fundamental synthetic chemistry, the culturing and co-culturing of cells using conditions closer to natural physiological environments, and in small-molecule assay development, have enabled the discovery of compounds that modulate challenging cancer-relevant targets and processes[12,13].

CTD^2 investigators are especially interested in projects involving targets such as transcription factors and processes such as gene regulation and cellular differentiation. For example, small-molecule probe-development projects are underway involving both transcription factors (including STAT3, C/EBP (β and δ)[8] and MYC) and chromatin-modifying enzymes (including histone methyltransferases and histone demethylases) that have been identified from genomic studies of cancer.

Probing genetic alterations in mouse models of human cancers

Genomic characterization of human cancers has revealed many genes that are altered. Transgenic or knockout mice that contain germline alterations in the candidate cancer gene can be used to assess oncogenic function. However, their generation and analysis precludes high-throughput evaluation of mutated genes. Transplantable mouse models offer the advantage of speed because genetic lesions are introduced into stem or progenitor cells that are then transplanted into recipient animals. Such models exist for a number of cancer types, including lymphoma, glioblastoma and carcinomas of the liver[19–21]. These models can be used to screen large numbers of genes for oncogenicity and acquired dependencies[22] and to determine the efficacy of small-molecule probes that have been optimized for animal testing.

Conclusions

The CTD^2 Network was formed by the NCI to serve as a link in the overall effort to discover safe and effective patient-based cancer drugs and to facilitate their clinical development through the identification of the genetic features of human cancers that predict drug efficacy, resistance mechanisms and clinical biomarkers. The Network aims to relate these features to their unique dependencies and to identify small molecules that target them, even when this entails hard-to-drug targets and processes—an empirical path that begins and ends with cancer patients.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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